

ANALYST:		VPDES NO	
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Parameter: *Escherichia coli*
Method: Colilert-18 or Colilert-24 MPN
05-06

METHOD OF ANALYSIS:

	IDEXX Colilert-18 or Colilert- 24 with Quanti-Tray®/2000
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- 1) Is media stored at 4 to 30°C without exposure to direct light? [7.1]
- 2) Is unopened media older than 12 months discarded? [7.1]
- 3) Is Quanti-Tray®/2000 (counting range of 2,419 per 100 mL) used for analysis? (**Note:** Dilutions are rarely necessary when this tray is used.) [Permit]
- 4) Is the appropriate fluorescence Quanti-Tray®/2000 comparator available and is it stored in the dark at 2-30°C? Expired comparators should be discarded. (**Note:** Expiration date is on back of comparator.) [7.2]
- 5) Is incubator capable of maintaining 35 ± 0.5°C? (**Note:** QuantiTray® is warmed during the sealing process which replaces the 20 min. waterbath incubation in the method.) [6.1]
- 6) Are only glassware or plasticware that will not autofluoresce used during this analysis? [4.4]
- 7) Are samples dechlorinated (if chlorine is present) and cooled to 10°C immediately after collection? [8.2 & 40 CFR Part 136.3 Table II]
- 8) Is sample holding time of 6 hours being met? [40 CFR Part 136.3 Table II]
- 9) Once Colilert Snap Pack is separated from strip, is it tapped to ensure that all of the powder is at the bottom part of the pack prior to opening it? [11.1.2]
- 10) Is powder always added to 100 mL of sample or sample dilutions? [11.1.4]
- 11) Is sample gently mixed after Colilert media is added and prior to pouring into Quanti-Tray®/2000? (**Note:** All media may not dissolve during mixing. It should, however, be completely dissolved after incubation.) [11.1.5]
- 12) Is sample incubated 18 – 22 hrs. for Colilert-18; or 22 – 28 hrs. for Colilert-24 at 35 ± 0.5°C with fluorescence determined immediately upon removal from incubator? [11.2]
- 13) Is fluorescence checked in a dark environment by holding a 6 watt UV lamp with a 365 nm wavelength three to five inches away from sample? **Note:** If a battery operated UV lamp is used, lamp should be only two inches from sample. [11.2]
- 14) If an effluent sample has a background color when collected, has the inoculated/incubated sample been compared to a control blank? **Note:** Blank consists of an aliquot of the same sample in the same type of container that has not been inoculated and was held at room temperature. [11.2]
- 15) For samples with *E. coli*, is fluorescence at least equal to the level of that in the comparator? (Typically the florescence will be much greater in positive samples.) [11.2]
- 16) Is MPN properly calculated using the IDEXX MPN table? [12.2]
- 17) Are blanks analyzed for each lot of media and Quanti-Tray® containers? [Permit]
- 18) Are all procedures carried out in a manner that will neither inhibit desired bacterial growth nor cause contamination? [Permit]
- 19) Are used Quanti-Trays® and sample collection containers to which Colilert-18 was added autoclaved at 121°C for 15-30 minutes at 15 pounds of pressure prior to disposal? [5.3]

Y	N

		Y	N
20)	Is lab glassware tested annually for inhibitory residues or is the detergent certified by the manufacturer to be free of inhibitory effects? [Permit]		
21)	If used, are all autoclaved materials identified with heat sensitive tape? [Permit]		
22)	If hot air sterilizing oven is used is it of sufficient size to prevent internal crowding, and constructed to give uniform and adequate temperatures of 170°C ±10°C? [SM 9030 B.2]		
23)	Is all glassware sterilized for at least 60 minutes at a temperature of 170°C? If it can be demonstrated with recording thermometers that temperatures are uniform, 160°C is adequate. [Permit]		
24)	Is method capable of demonstrating permit excursions? [Permit]		

PROBLEMS: